# Sodium-hydrogen exchange inhibition preserves ventricular function after ventricular fibrillation in the intact swine heart

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**Background:** We tested the hypothesis that sodium-hydrogen exchange inhibition attenuates ventricular dysfunction after ischemia-reperfusion injury in the intact porcine heart.

**Methods:** Twelve pigs (weight, 30-45 kg) were evenly divided into 2 groups. Baseline ventricular function studies were based on echocardiography, conductance, aortic flow, and left ventricular pressure. Animals were given vehicle (control) or benzamide-N-(aminoiminomethl)-4-(4-[2-furanylcarbonyl]-1-piperazinyl)-3-(methylsulfonyl)methanesulfonate (BIIB 513; 3 mg/kg administered intravenously). Ten minutes later, hearts were subjected to 75 seconds of ventricular fibrillation. After reperfusion for 40 minutes, function studies were repeated. Hearts were arrested and excised. Postmortem data included passive pressure-volume curves and myocardial water content.

**Results:** Preload recruitable stroke work was significantly decreased from baseline after ischemia and reperfusion in the control group (27.7 ± 2.5 vs 48.0 ± 5.6 mm Hg [± SEM], $P = .001$) but not in the BIIB 513 group (43.0 ± 5.8 vs 45.5 ± 4.1 mm Hg, $P$ = not significant). In vivo diastolic and postmortem passive left ventricular compliance were reduced after ischemia and reperfusion for control animals but remained unchanged for animals receiving BIIB 513. Time required to recover baseline blood pressure after ventricular fibrillation was significantly longer for control animals (159 ± 15 vs 88 ± 14 seconds [± SEM], $P = .008$). Myocardial water content (78.97% ± 0.94% vs 77.86% ± 0.46% [± SEM]) and normalized left ventricular mass (137.24 ± 6.17 vs 128.41 ± 1.96 g [± SEM]) were insignificantly increased in control animals.

**Conclusions:** Sodium-hydrogen exchange inhibition attenuates ventricular dysfunction after 75 seconds of ventricular fibrillation and 40 minutes of reperfusion. This family of agents might prove useful in patients with severe left ventricular dysfunction undergoing ventricular fibrillation for implantable cardioverter defibrillator testing.

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Implantable cardioverter-defibrillators (ICDs) are effective treatment for refractory malignant arrhythmias, decreasing the incidence of sudden arrhythmic death to 1% to 2% per year.[1-3] Insertion requires induction of ventricular fibrillation (VF) to measure defibrillation thresholds (DFTs). ICD recipients often have markedly decreased ventricular function caused by cardiomyopathy, infarct-related scars, or left ventricular (LV) aneurysms.[1] Although perioperative mortality for routine ICD insertion is low (0.5%-

5%), eligibility has been expanded to patients formerly thought to be too ill (eg, patients with ventricular arrhythmias awaiting heart transplantation or patients with ejection fractions [EFs] of ≤10% or less).[3,4] DFT testing in some of these patients is considered too hazardous and is deferred. A small study from our laboratory revealed no mean change in intraoperative EF after ICD testing,[5] but there was considerable individual variation between patients. Also, cardiovascular collapse has been reported during ICD insertion and can occur without warning.[6-8] Animal studies have demonstrated significant decreases in cardiac output after repeated short intervals of VF,[9] whereas longer periods of VF resulted in profound heart failure.[10]

A considerable body of literature supports the capacity of the sodium-hydrogen ion exchange inhibitors to attenuate ischemia-reperfusion injury after regional or global ischemia by using energy metabolites or infarct size as indices of ischemic damage. However, the ability of sodium-hydrogen exchange (NHE) inhibition to attenuate ventricular dysfunction after iatrogenic VF has not been reported. Inhibition of NHE during ischemia and reperfusion has been shown to attenuate myocardial damage,[11,12] reduce infarct size,[13,14] limit regional dysfunction,[15] and attenuate arrhythmias.[16] NHE protection after global ischemia has been studied as well in isolated heart preparations[17-19] and in the setting of orthotopic transplantation of hearts harvested after normothermic ischemia.[20-22] Cariporide, a selective NHE inhibitor, has been shown to improve regional LV function in patients with acute myocardial infarction undergoing percutaneous transluminal coronary angioplasty[23] and was well tolerated by patients in the GUARDIAN trial,[24] which concluded that NHE inhibition could be beneficial in patients undergoing bypass surgery after infarction.

Consequently, we explored the possibility of pharmacologic protection from ventricular dysfunction in the setting of iatrogenic VF. We investigated the use of one of the newer members of the NHE family, benzamide-*N*-(aminoiminomethl)-4-(4-[2-furanylcarbonyl]-1-piperazinyl)-3-(methylsulfonyl)methanesulfonate (BIIB 513), a selective NHE-1 inhibitor, untested in human beings. Reagents were obtained from Boehringer Ingelheim Pharma KG (Biberach, Germany). We used a pig model because of the similarity of the porcine myocardium to the human myocardium and its susceptibility to myocardial ischemia.[25]

## Materials and Methods

### Overview

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Twelve male domestic pigs weighing 32 to 45 kg were anesthetized with atropine sulfate (1-2 mg administered intramuscularly), ketamine hydrochloride (20 mg/kg administered intramuscularly), and acepromazine (0.5 mg/kg). They were intubated, mechanically ventilated, and maintained on isoflurane (1.5%-2.5%) mixed with 100% oxygen. A heating pad was used to maintain body temperature. Arterial blood gases and serum electrolytes were periodically checked to monitor oxygenation and optimize ventilation. During the experiments, 0.9% saline solution was administered through an 18-gauge angiocatheter in the femoral vein at 10 $mL \cdot kg^{-1} \cdot h^{-1}$ for the first hour and then decreased to 5 $mL \cdot kg^{-1} \cdot h^{-1}$ for the duration of the study, a protocol developed in collaboration with laboratory veterinarians at Columbia University.

After preliminary studies were performed in 2 animals to determine an interval of VF that would produce reproducible changes in ventricular function after 40 minutes of reperfusion, animals were divided into 2 groups (control group, n = 6; BIIB 513 group, n = 6). Baseline hemodynamic indices were taken before and after 75 seconds of VF and 40 minutes of reperfusion. Hearts were subsequently arrested and excised, and postmortem studies were performed.

### Study Protocol

***In vivo studies.*** While the electrocardiogram (ECG) and peripheral blood pressure were monitored with a fluid-filled catheter (model 7758, 8-channel recording system, Hewlett-Packard), a median sternotomy was performed. The hemiazygous vein (which drains directly into the coronary sinus in pigs) was ligated, and a longitudinal pericardiectomy was performed. A snare was placed around the inferior vena cava. After systemic heparinization (100 U/kg), a calibrated 5F micromanometer pressure catheter (Millar Instruments) and a 5F dual-field 12-electrode conductance catheter with reversed configuration allowing apical placement (Millar Instruments) were placed into the left ventricle through a 4-0 prolene purse-string suture in the LV apex. The position of the conductance catheter was verified by means of 2-dimensional epicardial echocardiography to ensure that the tip was through the aortic valve and that contact of the electrodes with the endocardium was avoided. Final position was adjusted by inspection of the individual segment pressure-volume loops. By using an oscilloscope x/y plotter (Hewlett-Packard), counterclockwise rotation of each of the 5 individual pressure-volume segment loops ensured that each was within the LV cavity. Before data collection, a 6-mL arterial blood sample was collected to measure blood resistivity with the rho cuvette by using the Leycom Sigma- 5 conductance module (Cardiodynamics DV). A pericardial well was created by sewing a plastic sheet to the pericardium. The free edges of the sheet were brought out and draped over the opened sternum. The well was used to hold water-soluble echocardiography gel (Ultraphonic scanning gel, Pharmaceutical Innovations, Inc) around the heart during echocardiographic measurements. The gel was removed during conductance measurements. A 16-mm A-series transit time ultrasonic flow probe (Transonic Systems, Inc) was filled with acoustic coupling gel and placed around the ascending aorta. The probe was connected to a dual-channel flowmeter (HT207, Transonic Systems, Inc). Finally, a pliable latex insulator was placed under the heart to limit parallel conductance.[26]

**TABLE 1. Hemodynamic data before and after ischemia and reperfusion**

| Group | Baseline HR (beats/min) | Baseline CO (L/min) | Baseline peak SBP (mm Hg) | Duration of VF (s) | Peak recovery pressure (mm Hg) | Time to recovery of baseline BP (s) | HR p 40 min (beats/min) | CO p 40 min (L/min) | Peak SBP p 40 min (mm Hg) |
|---|---|---|---|---|---|---|---|---|---|
| Control 1 | 72 | 2.4 | 75 | 75 | 110 | xx | 84 | 2.3 | 80 |
| Control 2 | 56 | 1 | 83 | 66 | 77 | 180 | 67 | 0.6 | 72 |
| Control 3 | 56 | 2.3 | 90 | 80 | 100 | 185 | 56 | 1.6 | 55 |
| Control 4 | 60 | 1.6 | 70 | 74 | 106 | 120 | 67 | 1.4 | 76 |
| Control 5 | 83 | 2.4 | 90 | 76 | 120 | 150 | 76 | 1.5 | 85 |
| Control 6 | 47 | 1.3 | 100 | xx | 147 | xx | 65 | 1.4 | 66 |
| Average | 62.33 | 1.83 | 84.67 | 74.20 | 110.00 | 158.75 | 69.17 | 1.47 | 72.33 |
| SD | 12.97 | 0.66 | 9.43 | 5.89 | 17.91 | 30.10 | 8.19 | 0.54 | 10.71 |
| SEM | 5.80 | 0.29 | 4.22 | 2.63 | 8.01 | 15.05 | 4.09 | 0.22 | 4.37 |
| BIIB 1 | 61 | 2.1 | 67 | 89 | 140 | 120 | 64 | 3.4 | 58 |
| BIIB 2 | 72 | 2.3 | 116 | 83 | 144 | 70 | 84 | 1.1 | 86 |
| BIIB 3 | 75 | 1.8 | 87 | 63 | 169 | 130 | 89 | 1.5 | 121 |
| BIIB 4 | 87 | 1.6 | 102 | 72 | 111 | 70 | 85 | 1.9 | 83 |
| BIIB 5 | 82 | 1.3 | 91 | 73 | 107 | 90 | 95 | 2 | 100 |
| BIIB 6 | 60 | 1.5 | 82 | 65 | 150 | 45 | 83 | 2.3 | 112 |
| Average | 75.40 | 1.82 | 92.60 | 76.00 | 134.20 | 87.50 | 83.40 | 1.98 | 89.60 |
| SD | 9.96 | 0.40 | 18.20 | 10.15 | 25.59 | 32.52 | 11.67 | 0.87 | 23.18 |
| SEM | 4.46 | 0.18 | 8.14 | 4.54 | 11.44 | 14.54 | 5.22 | 0.39 | 10.37 |
| *P* value | .10 | .83 | .47 | .99 | .076 | **.008** | **.03** | .18 | .18 |

Significant *P* values are shown in bold. *HR*, Heart rate; *CO*, cardiac output; *SBP*, systolic blood pressure; *BP*, blood pressure; *p* 40 min, after 40 minutes of reperfusion; *xx*, data unavailable.

Echocardiograms were obtained (Vingmed CFM 800, GE Medical) for LV cross-sectional short-axis measurements and for LV volume determination by using a hand-held 5.0-MHZ ultrasound transducer. The pericardial well was filled with scanning gel (Ultraphonic scanning gel, Pharmaceutical Innovations, Inc) to provide a standoff between the epicardium and the ultrasound transducer. Echocardiograms, LV pressure measurements, aortic flow measurements, and ECGs were obtained in the steady state. Display of the ECG and calibrated LV pressure on the echocardiography system facilitated synchronization of echocardiographic frames with physiologic events.

After collection of baseline hemodynamic data (Table 1), pressure-volume loops were generated during preload reduction through caval occlusion. Before inferior vena caval occlusion, a 1-V deflection mark was recorded on the echocardiogram and on the digital data acquired on the computer to allow the synchronization of the echocardiogram, ECG, LV end-diastolic pressure value, and aortic flow value. After recovery of baseline blood pressure, inflow occlusion was repeated during simultaneous LV pressure and LV short-axis echocardiography recording with the conductance catheter turned off. After collection of baseline hemodynamic data, epicardial pacemaker leads were connected to the anterior surfaces of the right and left ventricles. Animals were then given 50 mL of 0.9% saline solution with or without 3 mg/kg BIIB 513 according to protocols developed for this drug to provide adequate drug levels 10 minutes after bolus injection.[27] Ten minutes later, VF was induced for approximately 75 seconds with a 13-V 800-mA alternating current through a transformer (Archer AC adapter, Radioshack; Figure 1). VF was terminated on cardioversion with 50 J by using the internal paddles of a defibrillator module (Hewlett-Packard). If cardioversion resulted in asystole, animals were paced (Medtronic 5375) at 60 beats/min until hearts regained an independent organized rhythm. This occurred in 4 of the 12 animals evenly distributed between groups. One animal given BIIB 513 had a fine VF that was unresponsive to cardioversion or pacing. This animal could not be resuscitated and was excluded from the study. After cardioversion, animals were allowed to recover for 40 minutes, and hemodynamic data and echocardiograms were again collected. At the beginning and conclusion of the experiment, a 10 mL-bolus of hypertonic saline (5%) was infused directly into the pulmonary artery for measurement of parallel conductance, as described below.

***Postmortem studies.*** Hearts were arrested by means of infusion of 3°C University of Wisconsin solution into the aortic root after aortic crossclamping and transection of the inferior vena cava and left inferior pulmonary vein. Hearts were rapidly excised and placed in a 3°C ice slurry of University of Wisconsin solution. Postmortem pressure-volume relationships were obtained as follows. An 18-gauge anigocatheter connected to one port of a 3-way stopcock was placed through the previously performed LV apex purse-string suture. The left ventricle was sealed by placing atraumatic clamps at the atrioventricular groove approximately 2 mm above the mitral annulus. The clamp was positioned to minimize annular compression or other distortions of LV geometry that could induce artifacts.[28] LV pressures were measured through a second port of the stopcock by using the same 5F micromanometer, as previously used after recalibration. The third port of the stopcock was used for volume infusion. Before pressure measurements, all air was eliminated from the system. Volume was infused into the left ventricle in 5-mL increments, and micromanometer pressure was recorded at a sampling rate of 200 points per second by using an analog-to-digital conversion recording system

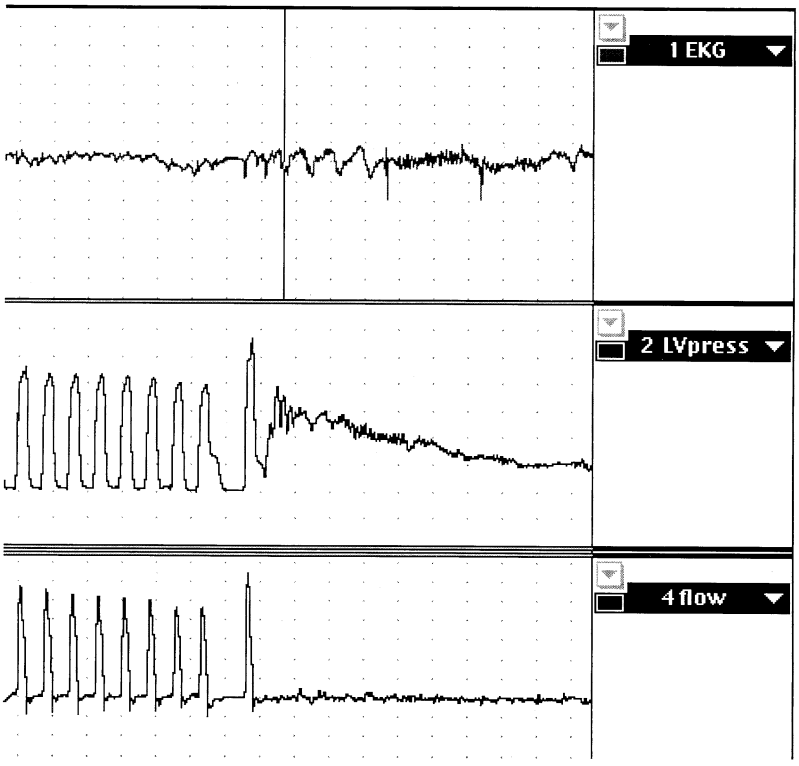


**Figure 1. Representative ECG, LV pressure tracing, and LV flow from a pig before and during induced VF.**

(MacLab, ADInstruments, Inc). Volume was infused until an LV pressure of 20 mm Hg was reached. The infused fluid was withdrawn and measured to determine whether leakage had occurred. In all animals, 95% or more of injected volume was recovered, and therefore no data were excluded from the pressure-volume analysis on this basis. This procedure was performed in duplicate for each excised heart.

After pressure-volume data were collected, the aorta, pulmonary artery, and atria were removed, and fat was trimmed. Excised hearts were placed on a preweighed piece of aluminum foil and weighed on an analytic balance (H16, Mettler Instruments Corp) to obtain heart wet weight (WW). The hearts were then dried to a constant weight in a 60°C oven for over 48 hours and reweighed to obtain heart dry weight (DW). The percentage of myocardial water content (MWC) was calculated as follows:

$$\text{MWC}\ (\%) = ([\text{WW} - \text{DW/WW}]) \times 100\%. \qquad (1)$$

## Data Acquisition and Analysis

Amplified pressure, conductance, and aortic flow were sampled at 200 Hz and transferred through a 16-channel analog-to-digital converter (MacLab, ADInstruments Inc) to a personal computer (IMAC, Apple Computer). Data were analyzed with the use of IGOR software (Wavemetrics, Inc). The total conductance signal was generated and processed in a Sigma 5 DF unit. The principles of conductance measurement of volume are described elsewhere.[29,30] Cumulative ejected volume determined by means of conductance and recorded during caval occlusion was plotted against the integrated aortic flow probe signal at each time point during ventricular ejection; gain was derived as the gradient of the linear regression. Correlation coefficients of the linear regression greater than 0.95 with a slope of the regression line within 0.3 of the line of identity (0.7-1.3) were accepted. Parallel conductance was calculated by a modification of the hypertonic saline injection method developed by Baan and associates.[30]

***Calculation of systolic function.*** Calculation of indices of systolic function during brief caval occlusion was performed with more than 8 consecutive cycles free of ventricular ectopy. All data were recorded with the ventilator off and measured at end expiration. For each cycle during caval occlusion, LV stroke work was calculated as the area of the pressure-volume loop. This was plotted against the end-diastolic volume. Preload recruitable stroke work (PRSW) was derived as the slope of the linear regression between stroke work and end-diastolic volume.[31] The greatest slope of the pressure-volume relationship throughout the cardiac cycle defined end systole, and the end-systolic pressure-volume relationship (ESPVR) defined maximum systolic elastance.[32]

***Calculation of diastolic function.*** The in vivo end-diastolic ventricular compliance curves were calculated in two ways. In the first method we used the conductance catheter to generate ventricular volumes as described above to create LV end-diastolic pressure-volume relationship (LV EDPVR) curves. In vivo LV EDPVR was obtained by superimposing the peak of the R wave of the ECG on the LV pressure and volume tracings. To enable comparison of animals of different sizes, LV raw volumes (V) were normalized to an average dry heart weight (W) of 28.74 g by using the following relationship:

**TABLE 2. Ventricular function before and after ischemia and reperfusion**

| Group | PRSW before (mm Hg) | PRSW after (mm Hg) | Vw before | Vw after | EF before (%) | EF after (%) | EF delta (%) | ESPVR before (mm Hg/mL) | ESPVR after (mm Hg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| Control 1 | 35 | 21 | 39.41 | 47.88 | xx | xx | xx | 1.19 | 1.6 |
| Control 2 | 61 | 30 | xx | xx | 41.3 | 32.9 | −20.3 | 1.9 | 1.3 |
| Control 3 | 41 | 25 | 8.43 | 16.35 | 52.6 | 49 | −6.8 | 4.6 | 1.2 |
| Control 4 | 62 | 36 | 56.72 | 34.18 | 22.8 | 19.3 | −15.4 | 3.1 | 2.34 |
| Control 5 | 35 | 22 | 26.7 | 26.34 | 31.5 | 28.7 | −8.9 | 1.4 | 1.26 |
| Control 6 | 54 | 32 | xx | xx | 35.6 | 26.3 | −26.3 | xx | xx |
| Average | 48.00 | 27.67 | 32.82 | 31.19 | 36.76 | 31.24 | −15.54 | 2.44 | 1.54 |
| SD | 13.79 | 6.13 | 24.38 | 8.94 | 12.83 | 12.40 | 6.17 | 1.42 | 0.47 |
| SEM | 5.63 | 2.50 | 9.95 | 3.65 | 5.24 | 5.06 | 2.76 | 0.63 | 0.21 |
| BIIB 1 | 27 | 24 | xx | xx | 37.9 | 37.1 | −2.1 | 0.93 | 0.75 |
| BIIB 2 | 53 | 32 | 62.22 | 41.86 | 40.9 | 32.7 | −20 | 1.83 | 1.46 |
| BIIB 3 | 49 | 59 | 26.85 | 14.32 | 47 | 37 | −20.7 | 1.7 | 3.1 |
| BIIB 4 | 42 | 39 | 3.64 | 4.74 | 18.1 | 19.4 | 7.2 | 1.68 | 2.27 |
| BIIB 5 | 48 | 45 | 16.04 | 25.87 | 30.6 | 33.5 | 9.5 | 1.53 | 2.68 |
| BIIB 6 | 54 | 59 | 51.1 | 27.68 | 25.8 | 36.6 | 41.9 | 3.43 | 6.38 |
| Average | 45.50 | 43.00 | 31.97 | 22.89 | 33.38 | 32.72 | 2.63 | 1.85 | 2.77 |
| SD | 10.01 | 14.24 | 24.31 | 14.09 | 10.59 | 6.79 | 23.19 | 0.84 | 1.96 |
| SEM | 4.09 | 5.81 | 10.87 | 6.30 | 4.32 | 2.77 | 9.47 | 0.34 | 0.80 |
| *P* value | .6991 | .0393 | NS | NS | NS | NS | .18 | .41 | .21 |

*Vw*, X intercept PRSW relationship; *xx*, data unavailable; *NS*, not statistically significant.

$$Vn = V(28.74/W). \quad (2)$$

In the second method of calculating in vivo end-diastolic ventricular compliance curves, we used echocardiography (as described below) to generate LV pressure-normalized LV area relationships.

Postmortem LV passive pressure-volume relationships were compared between groups by using methods previously described.[33,34] Briefly, a compliance curve was generated for each animal by plotting cumulative volume-injected and measured LV pressure. LV volumes were normalized to dry heart weight.

***Echocardiographic analysis.*** Images were digitized during real-time data acquisition by using the Vingmed CFM 800 echocardiography machine. End-diastolic short-axis cross-sectional images were planimetered by hand to obtain LV end-diastolic area.

### Statistical Analysis

PRSW was compared within groups before and after ischemia and reperfusion by using the Student *t* test. Comparisons between groups for hemodynamic data both before and after ischemia and reperfusion were made by using the Student *t* test. Pressure-volume curves and equations were generated by using Proc Mixed (SAS) methodology for repeated measurements. This method estimates the SEs by modeling the covariance structure of the repeated measures.[35,36] These measures are inherently correlated within the subject. Three of the more common covariance structures include *compound symmetry* for correlations that are constant for any 2 points in time, *autoregressive order one* for correlations that are smaller for time points further apart, and *unstructured*, which has no mathematic pattern within the covariance matrix. It was found that the autoregressive order one covariance structure provided the best fit for the postmortem data. This was discerned by means of comparing log likelihoods and the respective degrees of freedom for a given 2 models at a time.

For the in vivo data, a logarithmic transformation of all pressures was performed to exponentiate the data and provide the best fit. A significant interaction term was the requirement for statistically significant difference among the groups. For the postmortem data, a cubic polynomial function was fitted. *P* values were not corrected (eg, Bonferonni) for the multiplicity of testing. All data were analyzed by using SAS system software (SAS Institute Inc).

## Results

Data for changes in PRSW are shown in Table 2 and Figure 2. PRSW for control animals was significantly reduced from baseline after ischemia and reperfusion (27.7 ± 2.5 vs 48.0 ± 5.6 mm Hg [± SEM]; $P = .001$, paired Student *t* test) but not in the BIIB 513 group (43.0 ± 5.8 vs 45.5 ± 4.1 mm Hg, $P =$ not significant). PRSW after ischemia and reperfusion was significantly different between the 2 groups ($P < .04$, unpaired Student *t* test). The x intercept of the PRSW relationship did not change significantly after ischemia-reperfusion injury within or between groups. Data for changes in EF are shown in Table 2. Average baseline EF (36.8% ± 5.2% for the control group and 33.4% ± 4.3% for the BIIB 513 group [± SEM]) was insignificantly reduced by ischemia and reperfusion in both groups (31.2% ± 5.1% for the control group and 32.7% ± 2.7% the for BIIB 513 group [± SEM], $P =$ not significant). The average percentage change in EF showed trends toward decreased EF in control animals that fell short of statistical significance (−15.54% for the control group and +2.63% for the BIIB 513 group; $P = .178$, Mann-Whitney test). ESPVRs are reported in Table 2; preischemic values for the control and

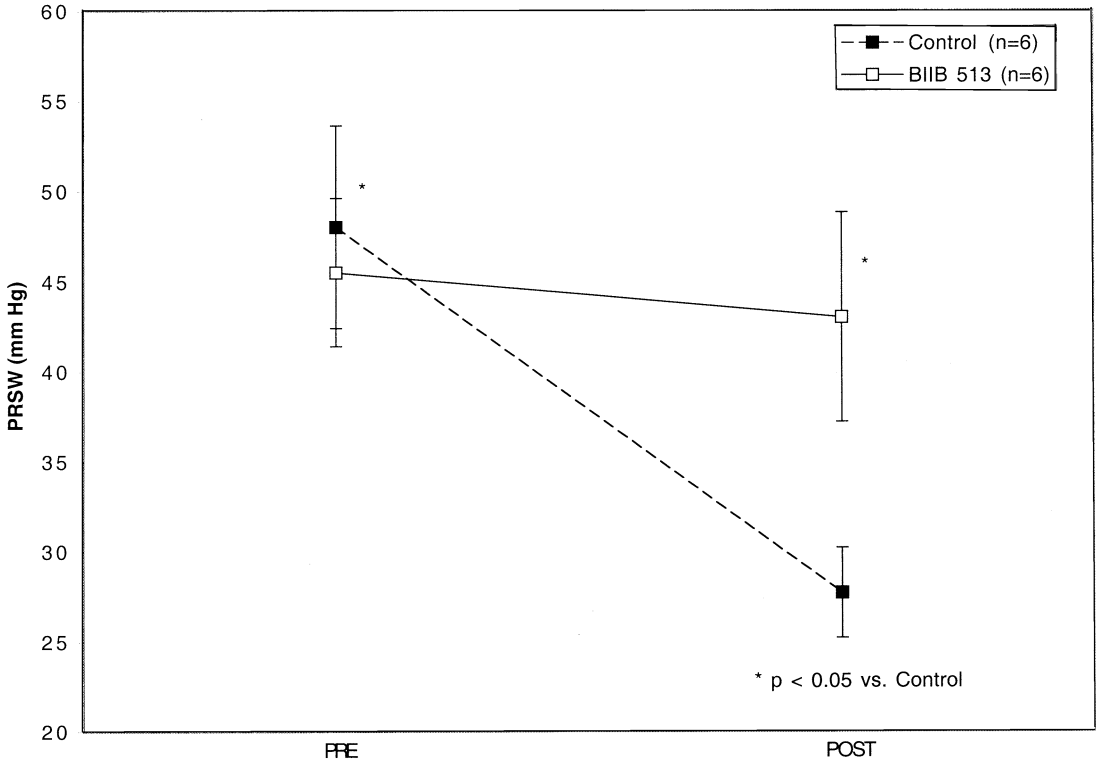


**Figure 2. Effect of BIIB 513 on PRSW after ischemia-reperfusion injury. The *ordinate* represents averaged PRSW in millimeters of mercury, and the *abscissa* represents the time point in the experiment: *PRE,* baseline levels; *POST,* 40 minutes of reperfusion after VF. *Filled squares* and the *dotted line* represent values for the control group, and *open squares* and the *solid line* represent values for the BIIB 513–pretreated group. SEs are represented by *brackets*. Differences in PRSW for control animals after 40 minutes of reperfusion are significantly different from those of control animals' baseline levels and BIIB 513–pretreated animals after 40 minutes of reperfusion ($P = .005$, analysis of variance).**

BIIB 513 groups (2.44 ± 0.6 vs 1.85 ± 0.3 mm Hg/mL [± SEM]) are not significantly different than postischemic values (1.54 ± 0.2 vs 2.77 ± 0.8), although trends suggested a decrease in ESPVR in the control group and an increase in the BIIB 513 group.

Data for changes in baseline hemodynamics are presented in Table 1. The average time required to recover baseline systolic blood pressure after VF was significantly longer for the control group when compared with that of the BIIB 513 group (158.75 ± 15 vs 87.5 ± 14.5 seconds [± SEM]; $P = .008$, Student *t* test). The average heart rate was also significantly decreased in the control group (69.17 ± 4 vs 83.4 ± 5.2 beats/min [± SEM]; $P = .03$. Student *t* test). Differences between the control and BIIB 513 groups in postischemic early peak systolic pressure (110 ± 8 vs 134 ± 11 mm Hg [± SEM], $P = .076$), cardiac output (1.47 ± 0.46 vs 1.98 ± 0.39 L/min [± SEM], $P = .18$) and peak systolic blood pressure after 40 minutes (72.3 ± 4.3 vs 89.6 ± 10.4 mm Hg [± SEM], $P = .18$) all reflected trends in decreased myocardial function in the control group but fell short of significance.

Data for in vivo end-diastolic pressure-volume (calculated by means of conductance) relationships are represented by Figure 3, *A*, and Table 3. Compliance reflected by the slopes of the LV compliance curves was significantly diminished from baseline after ischemia-reperfusion injury in the control group (0.021 ± 0.044 pre vs 0.039 ± 0.066 post; $P = .013$) but remained unchanged after ischemia-reperfusion injury in the experimental group (0.032 ± 0.004 pre vs 0.034 ± 0.0029 post; $P = .594$).

Data for in vivo end-diastolic pressure-area (calculated by echocardiography) relationships are represented by Figure 3, *B*, and Table 4. These data demonstrate a similar phenomenon, as observed when volume was measured by means of conductance (diminished compliance in the control group after ischemia-reperfusion injury); however, differences in the slopes of these compliance curves were not statistically significant: control pre 0.266 ± 0.05 vs control post 0.332 ± 0.080; $P = .475$; BIIB pre 0.295 ± 0.050 vs BIIB post 0.280 ± 0.060; $P = .850$).

Data for postmortem end-diastolic pressure-volume relationships are presented in Figure 4 and Table 5. The differ-

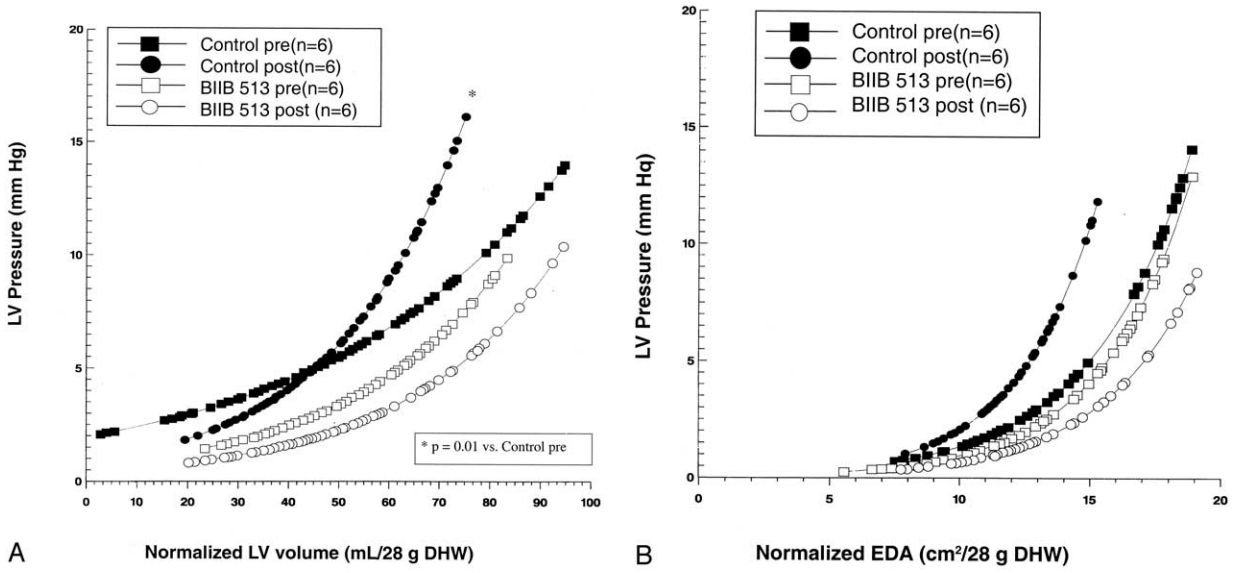


**Figure 3. A, Effect of sodium-hydrogen ion exchange inhibition on in vivo EDPVRs (by means of conductance) after ischemia-reperfusion injury. The *ordinate* represents end-diastolic LV pressure (in millimeters of mercury), and the *abscissa* represents average end-diastolic volume (in milliliters) generated by means of conductance and corrected for parallel conductance normalized to 28 g of dry heart weight *(DHW)*. *Filled symbols* represent control animals, and *open symbols* represent BIIB 513–pretreated animals. *Squares* represent baseline compliance, and *circles* represent compliance after ischemia and reperfusion. Values of the average slopes and SEs of the compliance curves are represented in Table 3. Ischemia and reperfusion in the control group led to a statistically significant increase in slope ($P = .0128$), demonstrating a decrease in compliance (smaller volumes at any given pressure), whereas for the BIIB 513 group, ischemia-reperfusion injury led to a statistically insignificant shift to the right (greater volumes at any given pressure). B, Effect of sodium-hydrogen ion exchange inhibition on in vivo end-diastolic pressure-area (by means of echocardiography) relationship after ischemia-reperfusion injury. The *ordinate* represents end-diastolic LV pressure (in millimeters of mercury), and the *abscissa* represents averaged end-diastolic area generated by means of echocardiography normalized to 28 g of dry heart weight *(DHW)*. *Filled symbols* represent control animals, and *open symbols* represent BIIB 513–pretreated animals. *Squares* represent baseline compliance, and *circles* represent average compliance after ischemia and reperfusion. Values of the average slopes and SEs of the compliance curves are represented by Table 4. Ischemia-reperfusion injury in the control group led to a statistically insignificant shift to the left (lesser volumes at any given pressure), whereas in the BIIB 513 group it led to a statistically insignificant shift to the right (greater volumes at any given pressure).**

**TABLE 3. Slopes of in vivo LV compliance curves (LV volume by conductance)**

| Group | Slope ± SE |
|---|---|
| Control before | 0.021 ± 0.0044* |
| Control after | 0.039 ± 0.0058 |
| BIIB before | 0.032 ± 0.0036† |
| BIIB after | 0.034 ± 0.0029 |

*$P = .0128$ versus control after.
†$P = .5939$ versus BIIB after.

**TABLE 4. Slopes of in vivo LV compliance curves (LV area by echocardiography)**

| Group | Slope ± SE |
|---|---|
| Control before | 0.266 ± 0.05* |
| Control after | 0.332 ± 0.08 |
| BIIB before | 0.295 ± 0.05† |
| BIIB after | 0.280 ± 0.06 |

*$P = .4753$ versus control after.
†$P = .8494$ versus BIIB after.

ence in slopes of the compliance curves (control: $y = -0.892 + 0.313\ vol - 0.002\ vol^2 + 0.0000315\ vol^3$ vs BIIB: $y = -0.621 + 0.195\ vol - 0.002\ vol^2 + 0.0000315\ vol^3$) was statistically significant and indicated that the control group had significantly lower volumes at any given pressure demonstrative of a loss in ventricular compliance.

Data for myocardial water content and normalized LV mass are presented in Table 6. Water content and normalized LV mass in the control group (78.97% ± 0.94% SEM and 137.24 ± 6.17 g SEM, respectively) was increased when compared with that in the BIIB 513 group (77.86% ±

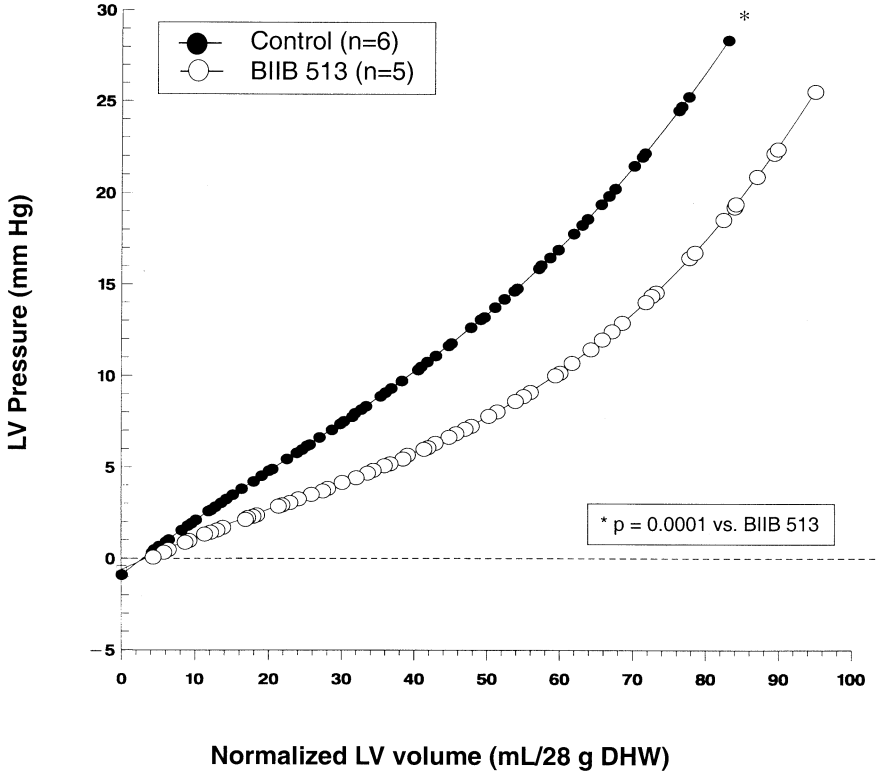


**Figure 4. Effect of BIIB 513 on postmortem passive pressure-volume relationship after ischemia-reperfusion injury. The *ordinate* represents averaged end-diastolic LV pressure, and the *abscissa* represents averaged LV volume normalized to 28 g of dry heart weight *(DHW)*. *Filled symbols* represent values for control animals, and *open symbols* represent values for BIIB 513–pretreated animals. Curves are fit as a cubic polynomial function. Control animals had an averaged postmortem compliance curve, with a statistically significantly steeper slope ($P = .0001$) representing a decrease in compliance.**

**TABLE 5. Formulas of postmortem LV passive pressure-volume curves**

| Group | Formula |
|---|---|
| Control | $y = -0.892 + 0.313vol - 0.002vol^2 + 0.0000315vol^3$* |
| BIIB | $y = -0.621 + 0.195vol - 0.002vol^2 + 0.0000315vol^3$ |

*$P = .0001$ versus BIIB.

0.46% and 128.41 ± 1.96 g, SEM) but did not reach statistical significance.

## Discussion

The present study indicates that NHE inhibition preserves LV myocardial contractility and attenuates loss in LV compliance after 75 seconds of VF. This is reflected by significant differences in both systolic (PRSW) and diastolic indices (in vivo and postmortem compliance curves) before and after ischemia-reperfusion injury for control animals, whereas experimental animals' indices were unchanged. The protective effect of NHE inhibition is also reflected by greater time to recovery of baseline systolic blood pressure in control animals after VF and trends toward diminished postischemic cardiac output and peak systolic blood pressure in control animals. Additionally, the protective effect is supported by statistically insignificant decreases in EF and ESPVR and increases in myocardial edema and normalized LV mass in control animals when compared with animals receiving BIIB 513.

This study was designed to assess the effects of ischemia and reperfusion. Although electrical defibrillation can cause direct myocardial cell injury, leading to postischemic myocardial dysfunction, significant injury only occurs at energy levels far exceeding those used to reverse VF in the current study.[37] Negligible or no injury has been reported after single countershocks at energy levels comparable with those used in our study.[38,39]

Clinically, NHE inhibition has been tested unsuccessfully for the prevention of myocardial infarction and mortality during percutaneous transluminal coronary angioplasty or surgical revascularization.[24] The present results suggest NHE inhibition could prove valuable for prevention of ventricular dysfunction during ICD insertion. The dura-

**TABLE 6. Animal and heart weights and myocardial water content**

| Group | BW (kg) | Wet HW (g) | Normalized WHW (g) | Dry HW (g) | MWC (%) |
|---|---|---|---|---|---|
| Control 1 | 45 | xx | xx | xx | xx |
| Control 2 | 36 | 153 | 154.51 | 28.46 | 81.40 |
| Control 3 | 35 | 125 | 149.31 | 24.06 | 80.75 |
| Control 4 | 43 | 145.4 | 131.08 | 31.88 | 78.07 |
| Control 5 | 40 | 127 | 121.10 | 30.14 | 76.27 |
| Control 6 | 35 | 104 | 132.84 | 22.5 | 78.37 |
| Average | 39.00 | 130.88 | 137.24 | 27.41 | 78.97 |
| SD | 4.34 | 19.19 | 13.79 | 4.00 | 2.09 |
| SEM | 1.77 | 8.58 | 6.17 | 1.79 | 0.94 |
| BIIB 1 | 45 | 151 | 129.47 | 33.52 | 77.80 |
| BIIB 2 | 30 | xx | xx | 30.95 | xx |
| BIIB 3 | 30 | 121.29 | 135.78 | 25.673 | 78.83 |
| BIIB 4 | 35 | 112 | 125.05 | 25.74 | 77.02 |
| BIIB 5 | 35 | 103 | 123.34 | 24 | 76.70 |
| BIIB 6 | 40 | 149 | 129.00 | 31.4 | 78.93 |
| Average | 35.00 | 121.82 | 128.41 | 27.98 | 77.86 |
| SD | 5.85 | 21.76 | 4.81 | 3.88 | 1.02 |
| SEM | 2.39 | 9.73 | 1.96 | 1.59 | 0.46 |
| *P* value | .32 | .73 | .19 | .64 | .48 |

*BW,* Body weight; *HW,* heart weight; *WHW,* wet heart weight; *MWC,* myocardial water content; *xx,* data unavailable.

tion of VF used in the present study is longer than that usually used during ICD insertion but not outside the range previously reported.[8] Furthermore, normal baseline ventricular function in the animals used here contrasts markedly with the diminished function commonly observed in ICD recipients.

Mortality and morbidity during ICD insertion can be due either to LV power failure or acute myocardial ischemia related to underlying coronary artery disease. Although not widely reported, DFT testing might be deferred in the highest-risk patients rather than risking death or serious injury as a result of testing. The present results suggest that NHE inhibition could provide increased safety for DFT testing in ICD recipients both by ameliorating power failure and limiting ischemic injury. In contrast to cariporide, BIIB 513 is a relatively novel agent that has not yet been used in clinical trials nor has its effect on DFT testing been investigated. This would need to be done experimentally before clinical use for this indication. We speculate that the observed protective effect of BIIB 513 is likely the result of limitations of increases of intracellular calcium concentration during ischemia, resulting in reduced reperfusion-induced calcium overload. Because animals were subjected to repeated episodes of limited ischemia during preload reduction from caval occlusions, it is worth considering the possibility of a role for ischemic preconditioning and/or myocardial stunning in our experiment, although significant differences between animals protected by NHE inhibition and controls suggests that the drug itself played an important role in observed differences in ventricular function after ischemia-reperfusion injury.

Our porcine model appears more vulnerable to myocardial ischemia than other experimental models. Variable degrees of potentially reversible systolic dysfunction have been reported after resuscitation from cardiac arrest in animal[40-42] and human[43] models; however, these ischemic intervals were considerably longer than 75 seconds. For example Kern[42] demonstrated severe ventricular dysfunction after 10 to 15 minutes of VF in intact swine hearts. Animals were resuscitated with dopamine and epinephrine. By 24 hours, invasive and noninvasive measures of systolic and diastolic LV function had begun to improve. At 48 hours, all measures of LV function had returned to baseline levels.

The present results confirm that load-dependent indices of systolic ventricular function, such as EF and ESPVR, are less sensitive indices than PRSW. Trends in EF and ESPVR in our study, however, reflect a pattern consistent with the PRSW data and together provide convincing evidence that there is a sustained decrease in systolic ventricular function after just 75 seconds of VF in the healthy pig heart.

Although ischemia and reperfusion generally results in impaired diastolic function in the intact heart,[42] some contradictory evidence has been described in isolated hearts and trabeculae.[41,44] A recent study in the isolated rat heart demonstrated that NHE inhibition ameliorated ischemic contracture, prevented postresuscitation diastolic dysfunction, and favored earlier return of contractile function after 25 minutes of iatrogenic VF.[45] Previous studies in pigs have suggested that diastolic dysfunction is worse immediately after resuscitation and improves throughout the reperfusion period.[42] Ischemic contracture has been reported as a cause of

reduced diastolic compliance, but the duration of ischemia in our study is too short to support this mechanism.

A comparison of the EDPVR from the postischemic in vivo studies and postmortem studies reveals curves of different shapes; in vivo data were fit to exponential curves, whereas postmortem data fit cubic functions better. This might be the consequence of differences in volume measurements (the conductance catheter was used in vivo, and direct volume infusion was used postmortem), or it might be the result of ischemic contracture resulting from the arrest or the ischemic period during calculation of the EDPVR. However, all postmortem studies were done within 15 minutes of cardiac arrest, whereas ischemic contracture does not occur in the pig heart, even after considerably longer periods of time.[48] A similar relationship in EDPVRs between the control animals and animals pretreated with BIIB 513 was evident in the postmortem studies compared with in the in vivo postischemic studies, suggesting that postmortem EDPVR accurately reflects in vivo changes in compliance.

Baseline effects of BIIB 513 administration on hemodynamics included a higher peak systolic pressure and heart rate compared with that in control animals, but these were not statistically significant (Table 1). The heart rate of experimental animals was also higher after 40 minutes of reperfusion, with comparable derived averaged stroke volumes (21 mL/beat for the control group vs 24.5 mL/beat for the BIIB 513 group). This difference might reflect an attenuated response to catecholamine stimulation in stunned myocardium. It might also be related to pre-existing differences between the 2 populations; the control animals had an average baseline heart rate of $62 \pm 6$ beats/min compared with $75 \pm 4.5$ beats/min in the BIIB 513 group, a difference that approached significance ($P = .10$, Student $t$ test).

The only animal in our study that could not be resuscitated after induction of VF had been given BIIB 513. As described above, the animal had a fine VF that was not terminated on multiple attempts at cardioversion and from which the animal could not be paced. This deserves further consideration, along with the effect of NHE inhibition on DFT testing.

Limitations of this study include the lack of documented serum drug levels (despite following protocols previously demonstrated to provide adequate drug levels) and the use of anesthesia not commonly used during ICD insertion. Additionally, this study was performed in healthy animals, while often in the clinical arena ICDs are inserted in patients with severe cardiomyopathies. Consequently, future studies should focus on enhancing clinical relevance of these data by using cariporide (HOE642), which has already been demonstrated to be safe in human subjects, and by better simulating the anesthetic conditions under which ICD insertion is performed.

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## References

1. Winkle RA, Mead RH, Ruder MA, et al. Ten year experience with implantable defibrillators. *Circulation.* 1991;84(Suppl):II-426.
2. Mirowski M. The automatic implantable cardioverter-defibrillator: an overview. *J Am Coll Cardiol.* 1985;6:461-6.
3. Spotnitz HM. Does ventricular fibrillation cause myocardial stunning during defibrillator implantation. *J Card Surg.* 1993;8(suppl):249-56.
4. Jeevanandam V, Bielefeld M, Auteri JS, et al. The implantable defibrillator: an electronic bridge to cardiac transplantation. *Circulation.* 1992;86(suppl II):II276-9.
5. Antunes ML, Spotnitz HM, Livelli FD, et al. Effects of electrophysiologic testing on ejection fraction during cardioverter/defibrillator implantation. *Ann Thorac Surg.* 1988;45:315-8.
6. Spotnitz HM, Hsu DT. Myocardial edema: importance in the study of left ventricular function. *Adv Card Surg.* 1994;5:1-25.
7. Spotnitz HM. Effects of ICD insertion. In: Spotnitz HM, editor. Research frontiers in implantable defibrillator surgery. Austin, Tex: RG Landes Co; 1992. p. 98-106.
8. Park WM, Amirhamzeh MMR, Jia CX, et al. Systolic arterial pressure recovery after ventricular fibrillation/flutter in humans. *Pacing Clin Electrophysiol.* 1994;17:1100-6.
9. Cole BN, Nicolosi AC, Uygur IJ, et al. Effects of simulated intraoperative electrophysiological testing on function of the canine left ventricle. *Ann Thorac Surg.* 1988;46:556-62.
10. Gazmuri R, Weil M, Bisera J, et al. Myocardial dysfunction after successful resuscitation from cardiac arrest. *Crit Care Med.* 1996;24: 992-1000.
11. Karmazyn M. The sodium-hydrogen exchange system in the heart: its role in ischemic and reperfusion injury and therapeutic implications. *Can J Cardiol.* 1996;12:1074-82.
12. Bethencourt D, Laks H. Importance of edema and compliance changes during 24 hours of preservation of the dog heart. *J Thorac Cardiovasc Surg.* 1981;81:440-9.
13. Klein H, Pich S, Bohle R, Wollenweber J, Nebendahl K. Myocardial protection by Na-H exchange inhibition in ischemic reperfused porcine hearts. *Circulation.* 1995;92:912-7.
14. Miura T, Ogawa T, Suzuki K, Goto M, Shimamoto K. Infarct size limitation by a new Na-H exchange inhibitor HOE642: difference from preconditioning in the role of protein kinase C. *J Am Coll Cardiol.* 1997;29:693-701.
15. Symons JD. Na-H exchange inhibition with cariporide limits functional impairment caused by repetitive ischemia. *J Cardiovasc Pharmacol.* 1998;32:853-62.
16. Scholz W, Albus U, Counillon L, et al. Protective effects of HOE 642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc Res.* 1995;29:260-8.
17. Shipolini AR, Galinanes M, Edmondson SJ, Hearse DJ, Avkiran M. Na/H exchange inhibitor HOE-642 imporves cardioplegic myocardial preservation under both normothermic and hypothermic conditions. *Circulation.* 1997;96(suppl 9):II-266-73.
18. Kevelaitis E, Oub E, Naissa A, Mouas C, Peynet J, Menasch P. Ischemic preconditioning with opening of mitochondrial adenosine triphosphate-sensitive potassium channels or Na/H exchange inhibition: which is the best protective strategy for heart transplants? *J Thorac Cardiovasc Surg.* 2001;121:155-62.
19. Moffat MP, Karmazyn M. Protective effects of the potent Na/H exchange inhibitor methylisobutyl amiloride against post-ischemic contractile dysfunction in rat and guinea-pig hearts. *J Mol Cell Cardiol.* 1993;25:959-71.
20. Martin J, Sarai K, Yoshitake M, et al. Orthotopic transplantation of pig hearts harvested after 30 min of normothermic ischemia: controlled reperfusion with blood cardioplegia containing the Na-H exchange inhibitor HOE 642. *Eur J Cardiothorac Surg.* 1998;14:607-14.

21. Kim YI, Herijgers P, Laycock SK, Van Lommel A, Verbeken E, Flameng WJ. Na/H exchange inhibition improves long-term myocardial preservation. *Ann Thorac Surg.* 1998;66:436-42.
22. Kim YI, Herijgers P, Van Lommel A, Verbeken E, Flameng W. Na/H exchange inhibition improves post-transplant myocardial compliance in 4-hour stored donor hearts. *Cardiovasc Surg.* 1998;6:67-75.
23. Rupprecht H-J, vom Dahl J, Terres W, et al. Cardioprotective effects of the Na/H exchange inhibitor cariporide in patients with acute anterior myocardial infarction undergoing direct PTCA. *Circulation.* 2000;101:2902-8.
24. Theroux P, Chaitman BR, Danchin N, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. *Circulation.* 2000;102:3032-8.
25. White FC, Blolor CM. The pig as a model for myocardial ischemia. In: Tumbleson ME, editor. Swine in biomedical research. Vol. 1. New York: Plenium Press; 1986. p. 481-90.
26. Cabreriza SE, Dean DA, Jia CX, Dickstein ML, Spotnitz HM. Electrical isolation of the heart. Stabilized parallel conductance for left ventricular volume measurement. *ASAIO J.* 1997;43:M509-14.
27. Gumina RJ, Buerger E, Eickmeier C, Moore J, Daemmgen J, Gross GJ. Inhibition of the Na/H exchanger confers greater cardioprotection against 90 minutes of myocardial ischemia than ischemic preconditioning in dogs. *Circulation.* 1999;100:2519-26.
28. Powell WJ, Bianco J, Freedberg LE, Dinsmore RE, Sanders CA, Daggett WM. Artifactual changes in apparent left ventricular compliance due to atrial clamping. *Appl Physiol.* 1970;78:515-8.
29. Baan J, Van der Valde ET, DeBruin HG, et al. Continuous measurement of left ventricular volume in animal and humans by conductance catheter. *Circulation.* 1984;70:812-23.
30. Baan J, Steendijk P, Koops J. Calibration and application of the conductance catheter for ventricular volume measurement. *Automedica.* 1989;11:357-65.
31. Glower DD, Spratt JA, Snow ND, et al. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. *Circulation.* 1985;71:994-1009.
32. Sagawa K. The ventricular pressure-volume diagram revisited. *Circ Res.* 1978;43:677-86.
33. Amirhamzeh MMR, Jia C-X, Park WM, Dickstein ML, Spotnitz HM. Systolic arterial pressure recovery after ventricular fibrillation in pigs. *Ann Thorac Surg.* 1994;58:1374-9.
34. Soto PF, Jia C-X, Carter YM, et al. Effect of improved myocardial protection on edema and diastolic properties of the rat left ventricle during acute allograft rejection. *J Heart Lung Transplant.* 1998;17:608-16.
35. Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS System for Mixed Models. Cary, NC: SAS Institute Inc; 1996.
36. Verbeke G, Molenberghs G. Linear mixed models for longitudinal data. New York: Springer-Verlag; 2000.
37. Xie J, Weil MH, Sun S, et al. High-energy defibrillation increases the severity of postresuscitation myocardial dysfunction. *Circulation.* 1997;96:683-8.
38. Doherty PW, McLaughlin PR, Billingham M, et al. Cardiac damage produced by direct current countershock applied to the heart. *Am J Cardiol.* 1979;43:225-32.
39. Kerber RE, Partins JB, Gascho JA, et al. Effect of direct-current counter-shocks on regional myocardial contractility and perfusion: experimental studies. *Circulation.* 1981;63:323-32.
40. Tang W, Weil MH, Sun S, et al. Myocardial dysfunction after successful resuscitation from cardiac arrest. *Crit Care Med.* 1993;21:1046-50.
41. Gazmuri RJ, Berkowitz M, Cajigas H. Myocardial effects of ventricular fibrillation in the isolated rat heart. *Crit Care Med.* 1999;27:1542-50.
42. Kern KB. Myocardial dysfunction after resuscitation from cardiac arrest; an example of global myocardial stunning. *J Am Coll Cardiol.* 1996;28:232-40.
43. Deantonio HJ, Kaul S, Lerman BB. Reversible myocardial depression in survivors of cardiac arrest. *Pacing Clin Electrophysiol.* 1990;13:982-5.
44. Gao WD, Altar D, Backx PH, et al. Relationship between intracellular calcium and contractile force in stunned myocardium: Direct evidence for decreased myofilament $Ca^{2+}$ responsiveness and altered diastolic function in intact ventricular muscle. *Circ Res.* 1995;76:1036-48.
45. Gazmuri RJ, Ayoub A, Hoffner E, Kolarova J. Successful ventricular defibrillation by the selective sodium-hydrogen exchange isoform-1 inhibitor cariporide. *Circulation.* 2001;104:234-9.
46. Kusuoka H, Porterfield JK, Wisman HF, et al. Pathophysiology and pathogenesis of stunned myocardium: Depressed Ca++ activation of contraction as a consequence of reperfusion-induced cellular calcium overload. *J Clin Invest.* 1987;79:950-61.
47. Krause SM, Jacobus WE, Becker LC. Alterations in cardiac sarcoplasmic reticulum calcium transport in the postischemic "stunned myocardium." *Circ Res.* 1989;65:526-30.
48. Hsu DT, Weng Z-C, Nicolosi AC, Detwiler PW, Sciacca R, Spotnitz HM. Quantitative effects of myocardial edema on the left ventricular pressure-volume relation. *J Thorac Cardiovasc Surg.* 1993;106:651-7.